MIXED MONOLAYERS OF LINEAR GRAMICIDINS AND PHOSPHOLIPID

Surface Pressure and Surface Potential Studies

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ABSTRACT The behavior of two gramicidins incorporated into lipid monolayers is analyzed on the basis of the force and surface potential area curves. It is shown that the position of the gramicidins (helical axis parallel or perpendicular to the interface) depends on the monolayer pressure and that these molecules are not miscible with dioleoylphosphatidylcholine. Surface potential measurements suggest the existence of a relationship between the single channel characteristics and the surface potential and indicate that the tryptophans are essential for lowering the lipid surface potential in agreement with the single channel behaviour of both gramicidin A and gramicidin M.

INTRODUCTION

It is now well established that the ionic channel of linear gramicidins (HCO—L$_1$Val—G$_2$ly—L$_3$Ala—D$_4$Leu—L$_5$Ala—D$_6$Val—L$_7$Val—D$_8$Val—L$_9$X—D$_{10}$Leu—L$_{11}$X—D$_{12}$Leu—L$_{13}$X—D$_{14}$Leu—L$_{15}$X—NHC_2H_4OH, where X is an aromatic residue) is very probably formed in a membrane by a head-to-head dimer of π^6_{DL} helices (Urry, 1971).

In this model the channel wall is built of the peptide backbone and the amino acid sidechains point outside the channel toward the lipid medium. It is also known that the conductance of the gramicidin channel can strongly depend on the nature of some sidechains. This was experimentally shown using analogues for which either residue 1 (Mazet et al., 1984; Barrett-Russel et al., 1986) or the aromatic residues X were varied. For the latter, two situations were observed. The first, obtained for GA (X = Trp) (Hladky and Haydon, 1972) and GT (X = Tyr) (Trudelle and Heitz, 1987), corresponds to channels the conductances of which are "high" and almost independent of the applied voltage, whereas the second, which is obtained for GM (X = Phe) (Heitz et al., 1982) and GTBzl (X = TyrBzl) (Daumas et al., submitted for publication), corresponds to "low" conducting channels with a potential-dependent conductance. The differences between the single-channel conductances of these two groups of gramicidins have been attributed to differences in the energy profiles of the channels (Heitz et al., 1986). For GA and GT, the binding step is the rate-determining process, whereas for GM and GTBzl, it is the translocation step. To elucidate the role of the aromatic sidechains, the four gramicidin analogues GA, GT, GM, and GTBzl were studied at the air-water interface (Van Mau et al., 1987). It was shown that, for pure gramicidin monolayers, the molecules are aligned with the helix axes parallel to the air-water interface with a molecular area of ~240 Å^2 which is compatible with either a single-stranded π^6_{DL} helix (Urry et al., 1971) or a double-stranded helix (Veatch et al., 1974). Furthermore, surface potential measurements made on these gramicidin monolayers showed similarities between GA and GT on one hand and between GM and GTBzl on the other. However, these experiments were carried out without lipids and thus do not take into account possible lipid-peptide interactions. Nevertheless, from the measurements that have been made, it appears that a relationship between the surface potential and the single channel behavior of the gramicidins may exist. Therefore we have undertaken a comparative study of lipid/gramicidin monolayers using one molecule from each group. In the present work we describe studies with GA and GM. The results will be discussed with reference to observations made on bilayers because, in the particular case of gramicidins, a monolayer can be considered as half a bilayer because of the head-to-head dimer structure of gramicidin channels.

EXPERIMENTAL

Gramicidin A was obtained as commercial gramicidin D (Sigma Chemical Co., St. Louis, MO) and recrystallized before use. Gramicidin M has the same origin as described in Heitz et al., 1982.

Dioleoylphosphatidylcholine (DOPC) was purchased from Sigma Chemical Co. and used without further purification, as it shows the same purity before and after purification.

Solutions of the gramicidins were prepared at room temperature by dissolution of 2 mg in 0.3 ml methanol (GA) or 0.7 ml chloroform (GM). After complete dissolution of the materials, the solutions were then diluted up to 1 ml by chloroform and methanol, respectively.

Solutions of lipid were prepared by dissolution of DOPC in chloroform-

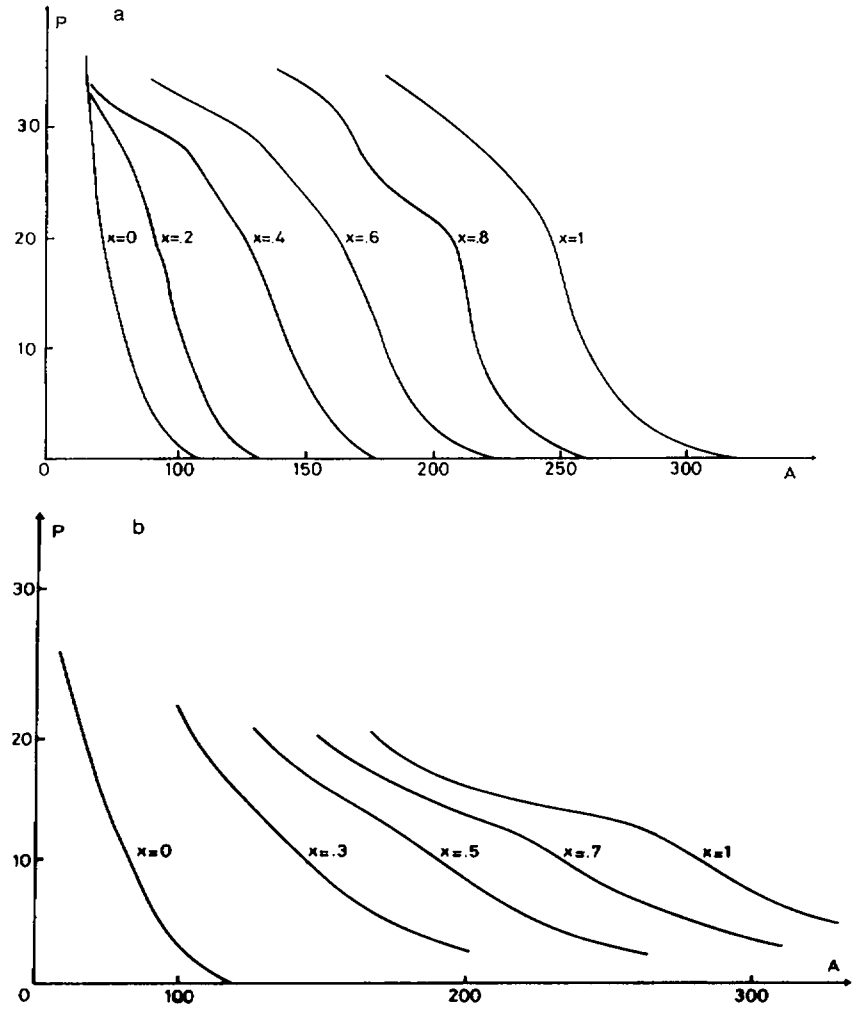

FIGURE 1 (*a*) Force-area curves at various GM/DOPC ratios (x). (*b*) Force-area curves at various GA/DOP ratios (x).

methanol 7:3 at the same molar concentrations as the gramicidin solutions, and the various gramicidin/lipid ratios were obtained by mixing the required volumes of the gramicidin and lipid solutions.

10–40 μl of the latter depending on the gramicidin/lipid ratio were spread on a Teflon Langmuir trough filled with threefold distilled water with a surface tension >72 dyn/cm to obtain a monolayer with an initial molecular density of $\sim 2.10^{-3}$ mol/Å^2.

All measurements were made at 24 ± 1°C and compression started at least 5 min after spreading. The films were then compressed continuously with a Teflon barrier at a compression rate of 5 Å^2/molecule per min.

Both surface pressure and surface potential variations were recorded simultaneously on a X-Y-Y' BD/91 recorder (Kipp & Zonen, Bohemia, NY).

All data reported here are the average of five measurements.

The surface pressures were measured using a tensiometer (Prolabo, Paris) based on the Wilhelmy method, and surface potential measurements were made using two identical Americium 241 air-ionizing electrodes which were placed on both sides of the barrier and connected to a voltmeter (model 619, Keithley Instruments, Inc., Cleveland, OH) with an impedance $>2.10^{13}$ Ω.

RESULTS AND DISCUSSION

Surface Pressure

The force area plots obtained at various GM/lipid ratios (x) are shown in Fig. 1. For $x = 0$, i.e., pure lipid, only one inflection at 40 dyn/cm (not shown in the figure) is detected and corresponds to the collapse pressure of the DOPC monolayer. For $x = 1$, i.e., pure GM, again only one inflection around 24 dyn/cm is observed and, as already shown in a preceding report, this pressure corresponds to the collapse pressure when the GM molecules are in a close-packed situation with their helical axes parallel to the interface (horizontal position) (Van Mau et al., 1987). When x is varied from 0.2 to 0.8, i.e., mixed GM-lipid monolayers, two distinct inflections can be detected and their corresponding pressures are given in Table I, where P_1 and P_2 refer to the low and high pressure inflections, respectively.

In Fig. 2 we have reported the variations of the molecular areas corresponding to P_1 and P_2 as a function of the GM/lipid ratio. Two possibilities can account for the linear variations of the molecular areas: either GM and DOPC are ideally miscible or they are not miscible at all. The second possibility requires constant pressures for the inflection points whereas, for the first one, continuous variations of P_1 and P_2 are expected. Examination of Table I shows that within the experimental precision P_1 and P_2 are constant for any x between 0.2 and 0.8. Therefore, on the

TABLE I
CHARACTERISTICS OF THE MIXED GRAMICIDIN A- OR GRAMICIDIN M-LIPID MONOLAYERS AT VARIOUS GRAMICIDIN/LIPID RATIOS

x	1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
P_1 for GM ($dyn\ cm^{-1}$)*	24	22	22	20	21	—	22	23	22	—
P_2 for GM ($dyn\ cm^{-1}$)*	—	—	31	28	29	—	28	29	29	—
P_1 for GA ($dyn\ cm^{-1}$)*	14	15	14	14	15	15	16	—	—	—
V_1 for GM (mV)‡	—	285	300	256	276	267	285	271	265	271
V_2 for GM (mV)‡	—	—	316	291	292	280	315	300	310	—

*Uncertainty, 1 dyn cm^{-1}.
‡Uncertainty, 10 mV. Inflection points were determined as the intersection of the tangents determined on the pressure-area curves on both sides of the collapse.

basis of Crisp's rule (Gaines, 1965), it can be stated that GM and DOPC are not miscible. This conclusion implies that when the monolayer pressure is increased above P_2 the GM molecules are squeezed out of the monolayer.

The finding of two inflections in the force-area curves also suggests that two different close-packed situations occur for GM and their corresponding molecular areas have been determined as the areas extrapolated at $x = 1$ of the variations of the mean molecular areas with x. On this basis P_1 and P_2 are associated to molecular areas of 244 and 191 $Å^2$, respectively. Assuming that GM adopts the π^6_{DL} helical conformation proposed by Urry (1971) with a monomeric length of ~15 Å, a molecular area of 244 $Å^2$ obtained for molecules aligned parallel to the interface leads to cross-section diameter of ~16.2 Å and thus to a cross-section area of 206 $Å^2$. This latter value is very close to the molecular area corresponding to P_2 (191 $Å^2$). Also this value rules out the possibility of double helical conformation (Veatch et al., 1974). Indeed, although both models are undistinguishable because of many similar physical characteristics (Wallace, 1986), the molecular area at P_2 is not compatible with the double-helical structure which requires a calculated molecular cross-section area half that of one monomer of a π^6_{DL} helix. Therefore P_1 and P_2 are attributed to the collapse pressures of the GM molecules with their helical axis, respectively parallel and perpendicular to the air-water interface. Further, as there is no reason to suspect any double helix–π^6_{DL} helix transition when going from "horizontal" to "vertical" molecules, the behavior of GM in mixed monolayers can be summarized as shown in Fig. 3.

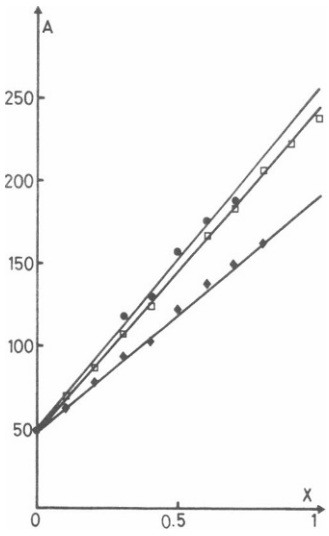

FIGURE 2 Variation of the mean molecular area corresponding to the inflection in the force-area curves of mixed GM or GA/DOPC monolayers. (♦) GM at P_1 = 22 dyn cm^{-1}. (□) GM at P_2 = 28 dyn cm^{-1}. (●) GA at P_1 = 13 dyn cm^{-1}.

Concerning the natural molecule GA, our data closely resemble those reported by Cornell et al. (1978) for GA–egg yolk lecithin mixtures, but these authors reported neither the low pressures ranges nor the high GA/lipid ratios ($x > 0.7$). Our own experiments at $x > 0.7$ showed poor reproductibility of force-area curves, so data is not shown in Figs. 1 *b* and 2 for $x > 0.7$. However, the collapse was observed for the same monolayer pressures for all values of x between 0.7 and 1. This suggests, as already mentioned by several authors, that GA can form large aggregates (Killian et al., 1985; Chapman et al., 1977; Kemp and Wenner, 1976). Examination of the force area plots of GA-lipid mixed monolayers (Fig. 1 *b* and in Cornell et al. [1978]) shows only one distinct collapse pressure corresponding to a GA molecular area of 250 $Å^2$, suggesting that the single collapse pressure in GA-DOPC monolayers is analogous to P_1 of GM-DOPC monolayers.

No collapse corresponding to P_2 could be detected for this system. This is possibly due to the slight increase of the bulkyness of the sidechains on going from Phe (GM) to Trp (GA), thus leading GA to nearly identical areas whatever the orientation is. That both GA and GM behave similarly was suggested by infrared information obtained on dried vesicles (Nabedryk et al., 1982) indicating that GA and GM in this lipid system adopt the same conformation characterized by an amide I band centered at 1,638 cm^{-1} and that both are oriented with their helical axis perpendicular to the plane formed by the lipid layers. As for GM, P_1 is independent of the GA/lipid ratio, and the corresponding molecular area varies linearly when x is increased from 0.2 to 0.7. This indicates nonmiscibility between GA and DOPC. Kemp et al. (1971) also observed for GA-lipid mixtures a transition in the force-area curves at 14 dyn/cm and an additivity of the molecular areas. However, the conclusion that GA and DOPC are immiscible conflicts with that of Cornell et al. (1978) who reported miscibility of GA with lipids. In our opinion, the difference

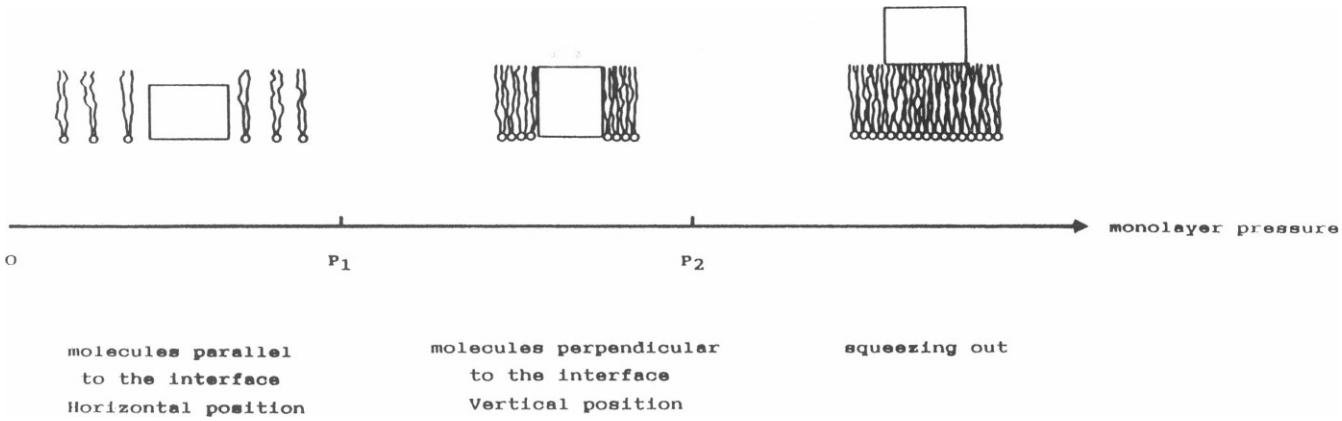

FIGURE 3 Schematic representation of the relative positions of GM and lipids depending on the monolayer pressure. P_1 and P_2 correspond to 22 and 28 dyn cm^{-1}, respectively.

in the two interpretations lies in the fact that these authors examined their experimental results at pressures which are higher than that of the collapse of the GA molecules.

This conclusion raises the following question. How can gramicidins form transmembrane channels as they are (*a*) not miscible with lipids, at least with DOPC, (*b*) squeezed out of the monolayer at pressures which are lower than that of a bilayer (~30 dyn/cm) (Blume, 1979) and thus they should not penetrate a bilayer? Several experimental situations may occur. (*a*) Gramicidin is present when the bilayer is formed. (*b*) Gramicidin is added to preformed bilayers. In the former case, owing to the high hydrophobicity it is reasonable to assume that the gramicidin molecules are pulled inside the core of the bilayer and the channel opening could correspond to the expulsion into the hydrophobic medium of two gramicidin molecules coming from each side of the bilayer. Thus, opening and closing of channels could be due to local fluctuations of the pressure leading to gramicidin molecules which fluctuate between the interface and the lipid core. In the second possibility, suggested by the observations made by Papahadjopoulos et al. (1973), who measured the penetration of GA in monolayers, due to the local fluctuations GA is first coadsorbed at the interface and then expulsed into the bilayer core leading to the same situation as previously described. It must also be mentioned that, when working on vesicles no transmembrane ion flux can be detected when the gramicidin is added to preformed vesicles whereas no gradient can be maintained when the vesicles are prepared in the presence of GA (Buster et al., 1988), thus providing a good argument indicating that GA does not penetrate highly condensed lipid bilayers.

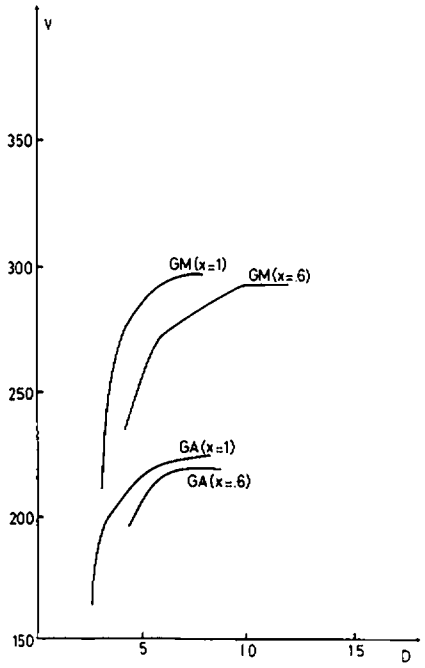

FIGURE 4 Variation of two gramicidin/lipid ratios of the surface potential as a function of the gramicidins molecular densities in the monolayer.

Surface Potential

On Fig. 4 we have reported the difference of surface potential as a function of the mean molecular density of the monolayer. Only two situations ($x = 0.6$ and 1) are shown for the sake of clarity, as those obtained for the other xs are similar to that of $x = 0.6$. Clearly, the curves obtained with GM show an inflection point at $\Delta V_1 \simeq 270$ mV and reach a plateau at $\Delta V_2 \simeq 300$ mV (see Table I for the detailed values at various x). By analogy with the surface pressure measurements ΔV_1 and ΔV_2 are associated with "horizontal" and "vertical" GM molecules, respectively. Examination of Fig. 4 also shows that for GA the surface potential corresponding to the plateau (ΔV_2) $\simeq$ 220 mV) is much lower than that of GM ($\simeq$300 mV), indicating that GA lowers the surface potential of the monolayer more strongly than GM.

CONCLUSION

The present investigations made on GA or GM/DOPC mixed monolayers reveal that these gramicidins are not

miscible with the lipid and that the descriptions of the gramicidin molecules are compatible with the π^6_{DL} helical structure proposed by Urry. Furthermore, the existence of two collapse pressures observed for GM indicates that the orientation of the synthetic analogue depends on the monolayer pressure.

Comparison of the surface potentials induced by the presence of GA and GM indicates that the presence of the natural gramicidin more strongly lowers the surface potential of a lipid monolayer than does GM. This observation, in addition to the published single-channel behavior of both GA and GM, suggests the existence of a relationship between the surface potential and the single-channel characteristics.

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